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Physiological and Biochemical Changes Associated With Virus-Induced Wilt of Tabasco Pepper.

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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ASSOCIATED WITH VIRUS-INDUCED WILT OF TABASCO PEPPER

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ABSTRACT

The physiological and biochemical changes associated with tobacco etch virus (TEV)-induced wilt of Tabasco pepper, Capsicum frutescens var. Tabasco, were studied. A marked release of electrolytes, indicative of permeability change, was detected in the roots of TEV-infected plants 24-48 hours before wilt symptoms occurred. Potassium and sodium ions were the main electrolytes that were lost to the surrounding solutions as a result of the altered permeability of the root cells. No such loss of electrolytes was obtained with roots of noninoculated control plants or with roots of TEV-susceptible pepper varieties which do not wilt as a result of infection. Permeability changes were also found to precede histological changes by 24-48 hours. A decrease in the respiratory rates of roots of infected Tabasco pepper plants was observed 12-24 hours after the initial change in permeability had occurred. The respiratory rate continued to decrease steadily thereafter, reaching a value about 50% of that of the control plants at the time of incipient wilt. No permeability changes were observed in systemically infected Tabasco pepper leaves during 144 hours after inoculation. An increased rate of respiration was detected in systemically infected leaves of Tabasco and 2 other TEV-susceptible pepper varieties at the time the external symptoms were apparent. Since the changes in permeability appeared to be

specific for TEV-infected Tabasco pepper roots, and since this preceded any other observable symptoms, a causal relationship between permeability changes and wilting is suggested.

The following biochemical changes were detected in root extracts of TEV-infected Tabasco pepper plants at the first day of wilt: a decrease in ascorbic acid content; a pronounced accumulation of polyphenols; an elevated enzymatic oxidation of ascorbic acid; and an increase in peroxidase activity. Polyphenol oxidase activity was similar to that of the control. Parallel tests made on root extracts of the other pepper varieties showed no significant differences between healthy and diseased plants.

The virus titer in roots of Tabasco pepper plants was similar to that in roots of other pepper varieties.

INTRODUCTION

The etiology of the wilt disease of Tabasco pepper was established in 1951 by Greenleaf (13, 14) who found that the disease was caused by a severe strain of tobacco etch virus (TEV).

The first visible symptoms following inoculation of Tabasco pepper with TEV are vein clearing and a faint mottling of the young leaves. These symptoms appear 4 days or more after inoculation, depending on environmental conditions and the virus concentration in the inoculum, and are followed within a few days by a lethal wilt (59). The root systems of plants that are starting to wilt have a rusty brown coloration. Phloem and cambium necrosis, extending in a ring around the xylem, is very characteristic in cross sections prepared from these roots (61, 62).

The wilt which occurs as a result of TEV infection of Tabasco pepper is a unique response of this variety. Other pepper varieties which are susceptible to TEV do not wilt when infected. The physiological and biochemical changes associated with the virus-induced wilt of Tabasco pepper were studied in an attempt to elucidate the nature of the factors responsible for the unique response of Tabasco pepper. These studies included: permeability and respiratory changes; changes in the activity of polyphenol oxidase and peroxidase; changes

in polyphenol content; changes in the activity of enzymes which oxidize ascorbic acid; and changes in ascorbic acid content.

The possibility that the virus titer in Tabasco pepper roots might differ from that in roots of other pepper varieties also was investigated.

REVIEW OF LITERATURE

The wilt disease of Tabasco pepper

Tabasco pepper, Capsicum frutescens L. var. Tabasco, is the major pepper variety used for the pepper pickling and hot sauce industry in Louisiana. Since Tabasco pepper is susceptible to the wilt disease caused by TEV, considerable losses in production have been experienced by growers for many years.

The disease was first studied in Louisiana by Person (40, 41, 42, 43) who, in a series of research notes, attributed the disease to certain fungi that were isolated from the wilted plants. He was never successful in determining the true nature of the pathogenic agent. Greenleaf (14), in Alabama, was the first to show conclusively that the disease was caused by a severe strain of tobacco etch virus. Sinclair et al (50), in 1959, showed that TEV was the cause of Tabasco pepper wilt in Louisiana. Horn and Sinclair (21), in a more detailed paper, showed that Tabasco pepper is susceptible to the virus at any stage of growth.

White and Horn's (61, 62) histopathological studies of infected roots at the first day of wilt revealed a phloem and cambium necrosis and degeneration of cortical cells and plastids. No phloem necrosis was detected in sections prepared from infected stem, petiole, or leaf tissue. The cells in these tissues, however, were increasingly

plasmolyzed as the disease progressed. Clogging of the xylem vessels was never observed in any of the tissues studied.

White (60), in further studies, showed that when Tabasco pepper scions were grafted to root stocks of other pepper varieties and then inoculated with TEV, wilt did not occur. In the reciprocal grafts, using Tabasco pepper as the rootstock, wilt occurred.

Tobacco etch virus, the causal agent of Tabasco pepper wilt, was first described on infected tobacco plants in Kentucky by Johnson (22). Bawden and Kassanis' (5) studies on the physicochemical properties of TEV revealed that the dilution end point, thermal inactivation point, and aging in vitro were 1/5000, 58°C for 10 minutes, and 13 days, respectively.

TEV is transmitted mechanically and by aphids and Bawden (4) listed as vectors: Aphis fabae Scop., A. rhamni Boyer, Macrosiphum gei Koch, Myzus circumflexus Buckson, and M. persicae Sulz. Laird and Dickson (30), in addition, reported Aphis gossypii Glover, A. spiraecola Patch, Macrosiphum solanifollii Thomas, and M. pisi Harris, as vectors.

The host range of TEV includes 83 plant species belonging to 15 families (19, 20). Forty-four of these species belong to the family Solanaceae.

Greenleaf (14) reported Chenopodium album L. as a local lesion host for TEV. He reported that circular lesions with necrotic

centers surrounded by concentric red rings and a yellowish halo developed in response to inoculation with TEV in extracts from tobacco leaves. Root and leaf extracts prepared from infected Tabasco peppers, however, failed to produce lesions on C. amaranticolor (60). The expressed juice from Capsicum frutescens L. has been shown by McKeen (36) to contain an inhibitor of TEV infection of Chenopodium sp. The physicochemical properties of the inhibitor indicate that it is a protein of low molecular weight, as it is thermolabile, passes through a cellophane membrane, and partially purified preparations give a positive Biuret test.

Physiology of virus-infected plants

"The effects of virus infection and multiplication on the physiology of host plants are still little understood. Compared with other specialities of virology the subject has been neglected." The preceding statements were quoted from the most recent review article on the subject written by Diener (7). In spite of the fragmentary and contradictory information in the literature, there are a few general principles on which most workers agree. The physiological changes most commonly associated with virus infection are summarized by Diener (7) as follows: (a) decreased photosynthetic activity; (b) increased rate of respiration; (c) accumulation of soluble nitrogen compounds, particularly amides; (d) increased activity of polyphenol

oxidase and accumulation of oxidized polyphenol derivatives; and (4) decreased activity of growth regulating substances.

Owen's (38) work on the effect of TEV infection on photosynthesis and respiration of tobacco leaves is the only report on the physiology of TEV-infected plants. Owen could not detect any alteration in the rates of photosynthesis and respiration of infected leaves until external symptoms were apparent. At that time photosynthesis decreased by 20% and respiration increased by about 40% over the control.

MATERIALS AND METHODS

A severe strain of TEV was maintained and increased in tobacco plants, Nicotiana tabaccum L. var. Havana 425. Systemically infected tobacco leaves harvested 10 to 14 days after inoculation were used as a source of the virus inoculum for all tests. The inoculum was prepared by grinding infected leaves in a mortar with water at a ratio of 1:5 (w/v). The leaves of the test plants to be inoculated were dusted with Carborundum and then rubbed with a cheesecloth pad previously dipped in the virus inoculum. Leaves dusted with Carborundum were rubbed with distilled water as a control.

The pepper varieties Capsicum frutescens L. var. Tabasco and California Wonder, and Capsicum annum L. var. Cayenne were used in these studies. The latter two varieties do not wilt as a result of TEV infection but develop vein clearing and mottling of the young leaves. Pepper seeds were sown in vermiculite kept moist with a modified Hoagland's nutrient solution. Seedlings in the 2-3 leaf stage were selected for uniformity and were transferred to washed white sand supplemented with nutrient solutions. The plants were then placed in a controlled chamber at 24-26°C with a 14-hour daily light period.

Seeds of Chenopodium amaranticolor Coste & Reyn., a local lesion host for TEV, were sown in vermiculite kept moist with nutrient solution. Individual seedlings were transferred to 8 inch pots

containing 4 parts loam, 2 parts peat, and 1 part sand. Vigorous growth was maintained throughout the experimental period so that the large, broad leaves that produce clear, sharp lesions would develop.

Methods for removal of the inhibitor in pepper extract

Since the expressed juice from pepper plants contains an inhibitor of TEV infection of Chenopodium sp., attempts were made to remove the inhibitor without interfering with virus infectivity. Various methods for clarifying crude juice were tested; these were adapted from Corbett's (6) work on the purification of potato virus X. Leaf extracts from TEV-infected tobacco leaves were included in the experiments, as a measure of the effect of the various treatments on virus infectivity. The crude juice obtained from Tabasco pepper roots at the first day of wilt and from infected tobacco leaves was subjected to centrifugation at 3000 rpm for 15 minutes in a SS-34 rotor of the model RC-2 Servall centrifuge. Aliquots of crude juice and the supernatant resulting from low speed centrifugation were tested for infectivity on C. amaranticolor.

One aliquot of the supernatant was mixed with activated charcoal (Merck N. F. Powder) at the rate of 0.1 g/ml. The mixture was stirred occasionally for 30 minutes and then filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtrate was then assayed for infectivity on C. amaranticolor. A second aliquot in an equal volume of chloroform was shaken for 5 minutes. The resulting

emulsion was broken by centrifugation, the light brown aqueous phase decanted and assayed for infectivity. Fifteen half leaves of C. amaranticolor were used for each treatment.

The infectivity of phenol-water extracts of TEV-infected tobacco leaves and Tabasco pepper roots also was investigated. The procedure used was essentially that developed by Schlegel (49). Plant tissues were ground in a chilled mortar with 4 times their weight of a cold 1:1 mixture of water-saturated phenol and 1% tetrasodium pyrophosphate solution. The resulting emulsion was then centrifuged for 5 minutes at 6000 rpm. The aqueous phase (containing the nucleic acid) was withdrawn and the excess phenol was removed by 3 ether washings. The nucleic acid was then precipitated by the addition of 2 volumes of cold 95% ethanol to one volume of aqueous phase. The precipitate was sedimented by centrifugation at 6500 rpm for 10 minutes, and the pellet containing the nucleic acid was resuspended in cold 1% K_2HPO_4. This material was tested for infectivity on C. amaranticolor and Tabasco pepper.

Virus titer in root extracts from 3 pepper varieties

Inoculation of a systemic host with serial dilutions of a virus preparation can also be used to measure virus concentration, although it is less sensitive than the local-lesion technique.

To estimate the virus titer of root extracts from the 3 pepper varieties, 5 plants of each variety were inoculated with a standard

inoculum. When the Tabasco pepper plants were just starting to wilt, root samples of equal weights were harvested from all 3 pepper varieties. Root extracts were prepared and 1:10, 1:100, and 1:1000 dilutions of the extracts were made. Twenty Tabasco pepper plants were used for the assay of virus infectivity of each dilution. Infectivity of each dilution was based on the number of wilted plants 6, 8, and 15 days after inoculation. These experiments were made twice.

Recovery of wilted Tabasco pepper plants

Greenleaf (14) showed that wilted Tabasco pepper plants would rapidly recover if their stems were severed and placed in water. White (59), using the same procedure, showed that plants which had been wilted for 12-24 hours and 5-12 days recovered within 10 hours, whereas plants which had been wilted for 25 days or more did not recover. Experiments were set up to study whether wilted plants would recover if placed in water after their root tips were excised. Infected Tabasco pepper plants which had been wilted for 1, 5, and 10 days were used. As controls, similar plants were either cut at the base of the stem and placed in water, or placed, intact, in water.

Permeability change determination

Twenty Tabasco pepper plants in the 4-5 leaf stage were removed from the sand and the roots were thoroughly rinsed. The roots of individual plants were then placed in 50 ml distilled water

of predetermined electrical conductivity. The plants were divided into 2 groups of 10 plants each. One group was inoculated by rubbing the 3 oldest leaves with the virus inoculum, and the other was rubbed with distilled water as a noninoculated control. Permeability changes were estimated from changes in the electrical conductivity of distilled water in which the roots were suspended. Measurements of the electrical conductivity of the ambient solutions were made at 12-hour intervals after inoculation with a conductivity bridge and the specific conductance in reciprocal ohms (mhos) was calculated. These experiments were repeated at least 5 times.

As a second control, permeability changes in systemically infected leaves of Tabasco pepper plants were determined. At 24-hour intervals after inoculation, 8 leaf discs, of 13 mm diameter, were cut from individual plants with a corkborer and then wrapped in cheesecloth bags bound with rubber bands. Five samples, from infected or healthy plants, were used for each determination. The bags containing infected and healthy tissues were placed individually in 250 ml flasks containing 50 ml of distilled water. The flasks were shaken for 10 hours and the electrical conductivity of the tissue bathing solutions was measured with a conductivity bridge and the specific conductance in mhos calculated.

As a third control, the permeability changes in roots of California Wonder, a variety of pepper that is susceptible to TEV but does not

wilt as a result of infection, were determined in parallel experiments using both California Wonder and Tabasco pepper.

Respiratory rate determination

The changes in the respiratory rates of leaves and roots of the 3 pepper varieties were determined by Warburg's direct method (52). Measurements of the respiratory rates were made with either a Precision constant volume respirometer or a Gilson differential respirometer.

To measure the respiratory rates of systemically infected and healthy leaves, samples of 7 leaf discs each were punched from individual plants with a No. 7 corkborer. The sampling process was standardized so that leaf samples from both infected and control plants would be of the same age. The fresh weight of leaf discs was determined so that the final results could be corrected accordingly. In some cases, the dry matter content was also determined. Four samples, from infected or control plants, were used for each determination. Immediately after weighing, leaf discs were placed in the main compartment of the manometric flask which contained 1 cc distilled water with 0.2 cc 20% KOH in the center well. All determinations of leaf respiration were made in the dark to prevent photosynthesis. The data were expressed as ul oxygen uptake per 200 mg fresh wt per hour. In some instances, when dry weight was determined, results were presented as ul O_2 per mg dry wt per hour.

For determining the respiratory rates of the roots, 300-500 mg (fresh wt) samples of root tissue were taken from individual plants and placed in the main compartment of the manometric flasks. The flasks contained 2 cc distilled water with 0.2 cc 20% KOH in the center well. In some experiments with Tabasco pepper roots, no water was added to the main compartment but 0.5 cc distilled water was placed in the side arm. Four samples from infected or control plants were used for each determination. The data were expressed as ul O_2 per 500 mg fresh wt per hour. In some experiments total nitrogen of root samples was determined and results were presented as ul O_2 per mg N per hour.

The bath temperature was 30°C and a thermal equilibration period of 20 minutes was allowed in all experiments.

Quantitative analysis

Total nitrogen was determined either by a modification of the direct Nesslerization method of Koch and McMeekin (27) or by the Micro-Kjeldahl method. For Nesslerization, 5 ml of the test sample were digested with 1 ml of 50% sulfuric acid and a quartz pebble. After digestion, samples were transferred to a 50 ml volumetric flask with about 35 ml of water. After swirling the contents of the flask, 12 ml of Nessler's reagent were added and the volume was made up to 50 ml with water. The mixture was allowed to stand for 10 minutes

for color development and the transmittancy was determined at 425 mu with a Bausch & Lomb Spectronic 20 colorimeter. A standard curve was prepared by adding Nessler's reagent to various dilutions of ammonium sulfate (0.4716 grams of ammonium sulfate diluted to one liter with 0.2N sulfuric acid containing 0.1 mg of nitrogen per ml). The procedure described by Hall and Hacskaylo (15) for total nitrogen determination by the Micro-Kjeldahl method was followed exactly.

Inorganic phosphorus was determined by a modification of the method of Fiske and Subbarow (10). Two ml of test sample were diluted to 14 ml with distilled water in a test tube. Four ml of ammonium molybdate reagent and 2 ml of amino-naphthol-sulfonic acid were added to the tube contents and then allowed to stand for 15 minutes before measurements of color intensity at 660 mu were made. A standard curve was prepared using monobasic sodium phosphate (0.089 gram dissolved and made up to one liter with water contains 0.02 mg phosphorus per ml).

Sodium (Na), potassium (K), and calcium (Ca) were determined by flame photometric techniques. Standard curves for Na, K, and Ca were made using a Beckman DB flame photometer. Sodium was determined at 589 mu with a slit width of 0.05 mm; potassium at 767 mu and slit width of 0.20 mm; and calcium at 423 mu and a 0.05 mm slit width. Fuel settings of 15 psi of oxygen and 3 psi of acetylene were used for all tests. The amount of each cation in test samples was determined by interpolation in the standard curve.

Chlorogenic acid was determined by paper chromatographic separation followed by ultraviolet spectrophotometric assay using the procedure described by Lee and LeTourneau (31). Chlorogenic acid was located on the chromatograms by examination in ultraviolet light. The position of the blue fluorescent spot of chlorogenic acid was verified by including known chlorogenic acid alongside the unknown. The fluorescent spot of chlorogenic acid was cut out, eluted in 2 extractions with 70% ethanol, and an aliquot of the eluates was used for absorption measurements at 325 mu in a Beckman DB spectrophotometer. A standard curve was prepared by following the same procedure with known amounts of chlorogenic acid. The amount of chlorogenic acid in the unknown was determined by interpolation in the standard curve.

Total orthodihydric phenols were determined with the Arnow reagent using the procedure of Johnson and Schaal (24).

Ascorbic acid was determined by a modification of the method of Roe and Kuether (46) using the 2,4-dinitrophenyl-hydrazine reagent. The modified method described by Hall and Hacskaylo (15) was followed exactly. In some experiments, ascorbic acid in 3% (w/v) metaphosphoric acid extracts was determined by Morell's (37) method using the indicator 2:6 dichlorophenol-indophenol.

Enzyme assays

In order to study the changes in the activity of soluble oxidases in roots of the 3 pepper varieties, tissue was homogenized using a

standard procedure. Three g samples (fresh wt) of root tissue were homogenized in 10 ml 0.15 M phosphate buffer, pH 6.0, in a Servall Omni-mixer, at 11,000 rpm for 2 min. The resulting homogenate was strained through 4 layers of cheesecloth and the filtrate, which will be referred to as root extract, was collected and placed in an ice bath until used. Boiled root extracts served as checks. Three samples from infected or control plants were used for each determination.

Polyphenol oxidase and the enzymatic oxidation of ascorbic acid were determined manometrically with a Gilson differential respirometer. Two ml of root extract were placed in the main compartment of the manometric flasks which contained 0.2 ml of 20% KOH in the center wells. One-half ml of 0.01 M chlorogenic acid or 0.028 M ascorbic acid in 0.15 M phosphate buffer, pH 6.0, was added to the side arms. Duplicate or triplicate flasks were used for each sample. The flasks were equilibrated at 30°C for 20 min, the manometers were closed, and 3 readings were made at 10-min intervals beginning at zero time. The side arm contents then were tipped into the main compartments and readings were continued for an additional 40 min.

Peroxidase was determined using a slight modification of the method described by Hamptom (16). Three ml of 0.05 M pyrogallol in 0.015 M phosphate buffer at pH 6.0 plus 0.2 ml of root extract in a colorimeter tube were adjusted to 0 optical density at 420 mu using a Bausch & Lomb Spectronic 20 colorimeter. One-half ml of 1% H_2O_2

was added and the tube was inverted to mix the contents and reinserted in the colorimeter. The time required for the optical density to increase from 0.1 to 0.3 was measured with a stopwatch. The enzyme activity was assumed to be inversely proportional to the time required for the change in the optical density.

EXPERIMENTAL RESULTS

Infectivity tests with root extracts

No lesions were produced when pepper root extract was rubbed onto Chenopodium leaves, whereas tobacco leaf extract produced abundant lesions (Fig. 1).

Various methods of clarification were applied to the crude extract of Tabasco pepper root in an attempt to remove the virus inhibitor which prevents TEV infection of C. amaranticolor. Data obtained from these experiments are summarized in Table 1. None of these succeeded in eliminating the inhibitor without loss of virus. Results obtained with tobacco leaf extract (Table 1, Fig. 2) indicated that charcoal adsorption and chloroform emulsion treatments either decreased or destroyed virus infectivity. Low speed centrifugation, however, resulted in only a small loss of infectivity (Table 1).

Phenol-water extracts of TEV-infected Tabasco pepper roots and tobacco leaves were not infective when assayed on Tabasco pepper. In infectivity tests using C. amaranticolor, 1-2 lesions were produced. Extracts prepared from comparable tissues ground in 1% K_2HPO_4 produced from 50-80 lesions per half-leaf.

In order to determine the virus titer in the roots of the 3 pepper varieties, serial dilutions of root extracts were prepared from these

Table 1. The number of lesions produced on Chenopodium amaranticolor leaves inoculated with crude and clarified extracts of TEV-infected tobacco leaves and Tabasco pepper roots.

Clarification procedure	No. of lesions per half-leaf[a] Source of virus: Tobacco leaves	Tabasco pepper roots
Crude juice	60	0
Low speed centrifugation[b]	50	0
Charcoal absorption[c]	3	1
Chloroform emulsion[d]	0	0

[a]Each figure is average of 15 replications.

[b]Supernatant

[c]Filtrate

[d]Aqueous phase

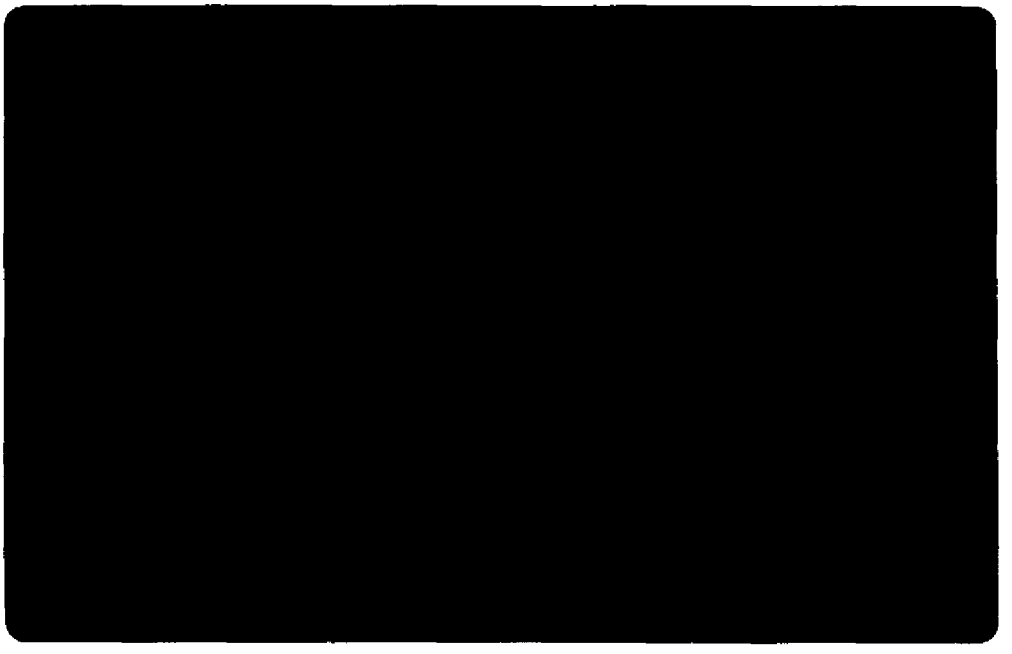

Figure 1. Local lesions produced on an inoculated leaf of Chenopodium amaranticolor by juice from TEV-infected tobacco leaves (left half) and TEV-infected Tabasco pepper roots (right half).

Figure 2. The effect of charcoal adsorption treatment on the infectivity of an extract of TEV-infected tobacco leaves determined by local lesion production on C. amaranticolor. Right half leaf: inoculated with a crude extract; left half: inoculated with filtrate obtained with charcoal treatment.

varieties. The infectivity of each dilution was assayed on 20 Tabasco pepper plants and estimation of the virus titer was based on the number of assay plants that had wilted 6, 8, and 15 days after inoculation. Infectivity of the 1/10 and 1/100 dilutions of all 3 varieties was very similar for all 3 time periods. No infection was obtained with 1/1000 dilutions of any of the varieties (Table 2).

Recovery of wilted Tabasco pepper plants after excision of root tips

Tabasco pepper plants which had wilted 1, 5, and 10 days previously, recovered when their root tips were excised and the plants were placed in water. Corresponding plants which were cut at the base of the stem recovered in a shorter period of time. Wilted plants which were removed from the sand and placed in water without excision did not recover.

Permeability and respiratory changes

Permeability changes in the roots of TEV-inoculated and control Tabasco pepper plants were determined at 12-hour intervals during the 144 hours after inoculation. A marked release of electrolytes, indicative of permeability change, was detected 24-48 hours before the inoculated plants started to wilt (Fig. 3). The loss of electrolytes increased steadily thereafter. No such release of electrolytes was obtained with noninoculated control plants.

Table 2. Assay of virus infectivity of serial dilutions of root extracts from 3 pepper varieties on Tabasco pepper plants.

Source of root extract	Dilution	No. of wilted Tabasco plants[a] Days after inoculation 6	8	15
Tabasco	1/10	12	20	20
	1/100	4	15	17
	1/1000	0	0	0
Cayenne	1/10	14	20	20
	1/100	3	15	16
	1/1000	0	0	0
California Wonder	1/10	12	20	20
	1/100	3	14	16
	1/1000	0	0	0

[a]Twenty Tabasco pepper plants were used for the assay of each dilution.

Permeability changes in systemically infected leaves of Tabasco pepper plants were determined as a second control. No differences in permeability were detected between systemically infected and healthy leaves during the 144 hours after inoculation (Fig. 3).

As a third control, permeability changes in the roots of TEV-inoculated and control California Wonder plants, a variety of pepper that does not wilt as a result of infection, were determined. Results obtained from these experiments showed no loss of electrolytes from either the inoculated or the noninoculated control plants (Fig. 4).

In order to provide more detailed information, qualitative and quantitative determinations of substances and electrolytes released from the roots of inoculated Tabasco pepper plants were made. At the time the initial increase in electrical conductivity of the ambient solutions occurred, these solutions were analyzed for total nitrogen (N), inorganic phosphorus (P), calcium (Ca), potassium (K), and sodium (Na). The results obtained from these tests showed that K and Na but not N, P, or Ca were present in the ambient solutions at the time of initial increase in conductivity. The ambient solutions in the case of inoculated plants contained five times as much K and three times as much Na as the control (Fig. 5). From 24-48 hours later, when the inoculated plants had started to wilt, still higher values of K were measured. No further increase in Na concentration occurred.

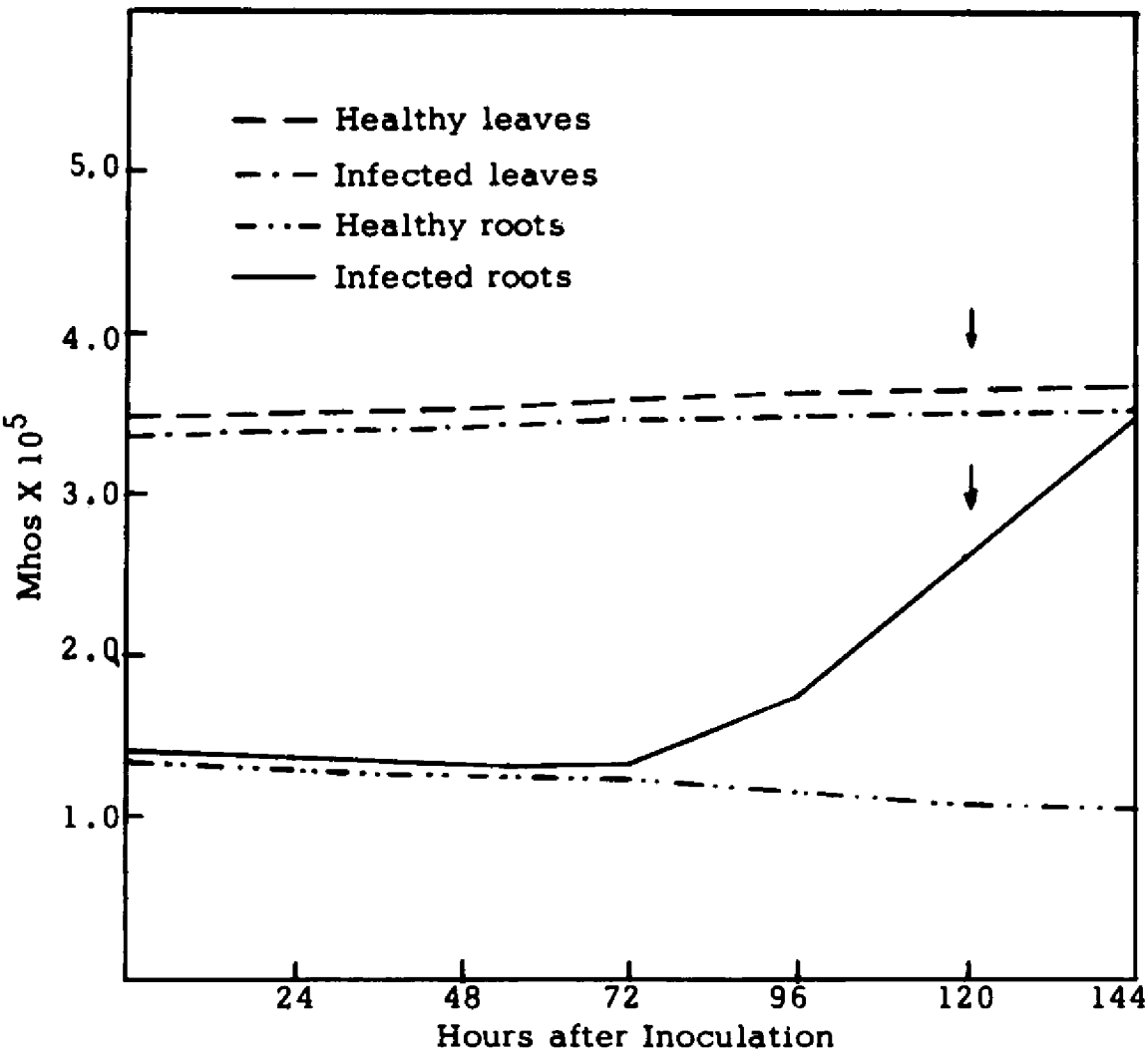

Figure 3. Changes in the electrical conductivity of the tissue bathing solution of leaves and roots of TEV-infected and control Tabasco pepper plants during the 144 hours after inoculation. The arrow refers to the time at which plants began to wilt.

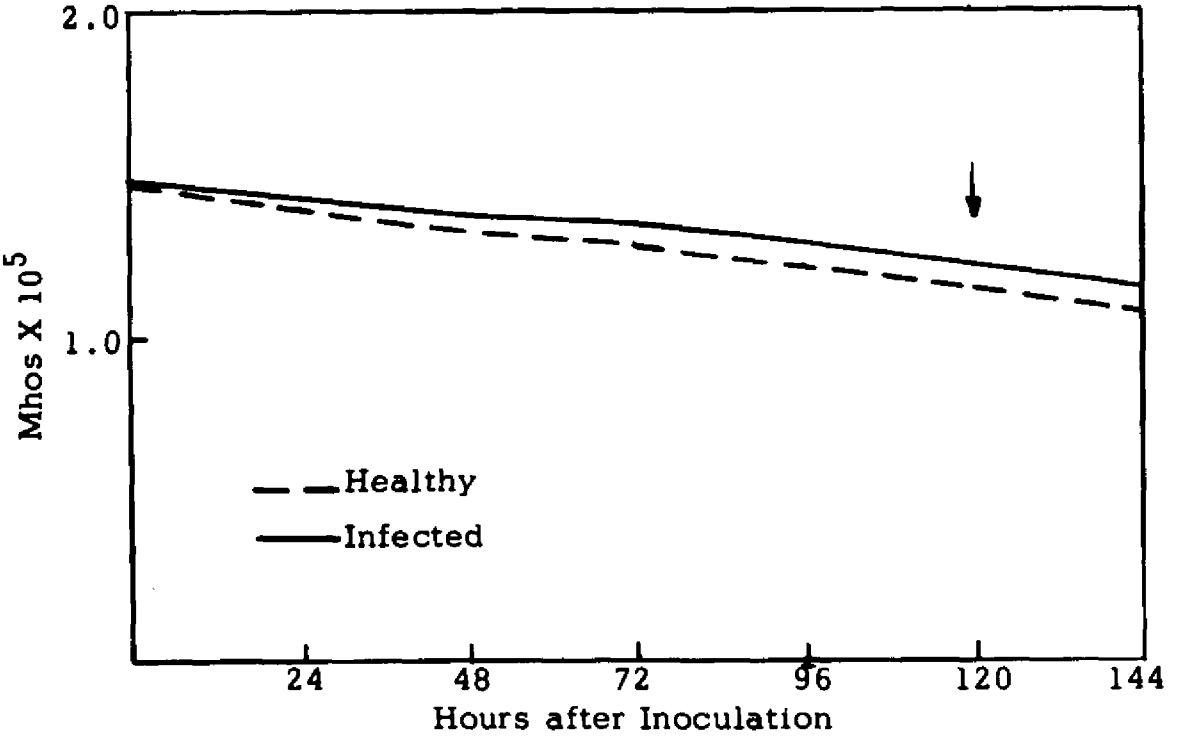

Figure 4. Changes in the electrical conductivity of the ambient solutions of roots of TEV-infected and control California Wonder pepper plants during the 144 hours after inoculation. The arrow refers to the time at which vein clearning and mottling symptoms appeared on young leaves.

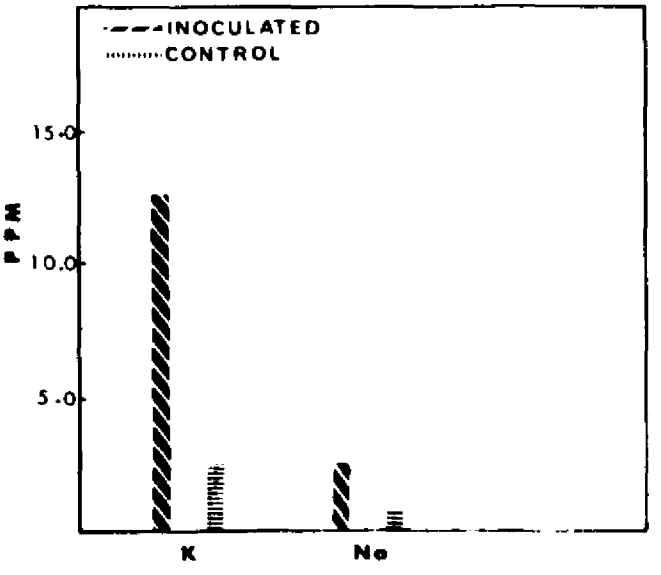

Figure 5. The amounts of K and Na detected in the ambient solutions of roots of TEV-infected and control Tabasco pepper plants at the time of initial change in permeability.

It was of interest to determine if TEV was present in the root tissues at the time the initial increase in permeability occurred. Infectivity tests were made on root extracts prepared at this time. Ten healthy Tabasco pepper plants were inoculated with the root extracts and data were recorded 10 and 15 days after inoculation. The results obtained from these experiments showed that 5 plants had wilted after 10 days and a total of 7 plants had wilted after 15 days. Thus, the virus was present in the roots at the time permeability change was first detected.

White and Horn (62) demonstrated cambium and phloem necrosis in the roots of wilted Tabasco pepper plants. To relate these histological changes to the permeability changes, stained sections were prepared from roots of both inoculated and control plants at the time the initial increase in permeability occurred. The procedure described by White and Horn (62) was used for preparing the sections. Stained sections were prepared from comparable tissues at the first day of wilt as a control. No phloem or cambium necrosis could be found at the time of initial electrolyte loss (Fig. 6). However, cambium and phloem tissues were disorganized and discolored at the first day of wilt (Fig. 6).

The changes in the respiratory rates induced by TEV infection in Tabasco pepper roots were measured. To relate changes in respiration, if the latter could be detected, to the permeability changes which

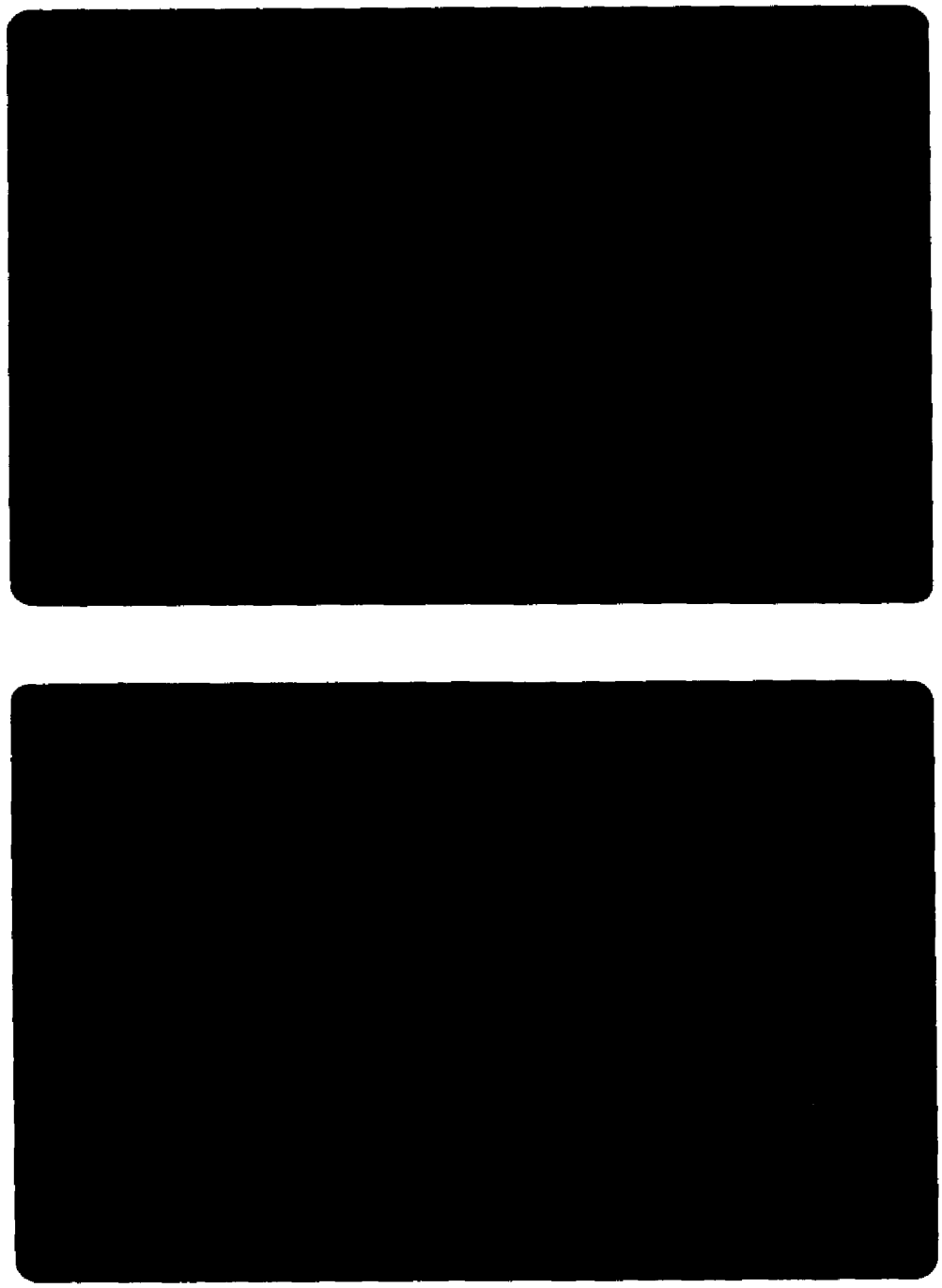

Figure 6. Cross sections of TEV-infected Tabasco pepper roots.
Upper figure: Section prepared at the time of initial increase in permeability.
Lower figure: Section prepared at the first day of wilt.

had been found to take place, root tissues to be studied were sampled as follows: immediately after each measurement of the electrical conductivity of the ambient solutions, representative root samples were harvested from both inoculated and control plants and the respiratory rates were determined. By this procedure, permeability and respiratory determinations were made on the same root system, thus providing a means for measuring the respiratory rates of roots whose condition of permeability change was known. The results obtained from these experiments showed no changes in the respiratory rates up to the time permeability change first became pronounced. However, 12-24 hours later, a decrease in oxygen uptake of the roots of inoculated plants was detected. The respiratory rate continued to decrease steadily thereafter, reaching a value of about 50% of that of the control at the time of incipient wilt (Fig. 7a).

Wheeler and Black (56, 57) detected an increase in respiratory rates that followed permeability changes in victorin-treated oat tissues. These authors, in an attempt to relate permeability changes to respiratory changes, suggested that as a result of increased permeability of the membranes, salts and other materials leaking from the vacuole and coming into contact with the mitochondria might account for the observed increase in respiration.

Since the roots used for respiratory rate measurements were bathed in distilled water, as described before, salts leaking from

the vacuole as a result of increased permeability of the membranes would be leached into the surrounding solutions. Thus, the possibility still existed that there was an increase in the respiratory rate, as suggested by Wheeler and Black (57), but that this might not be detected due to the experimental conditions. To investigate this possibility, determinations of permeability and respiratory changes were made on 2 separate groups of plants of the same age which were maintained under the same environmental conditions. For respiratory change determinations, the plants were kept in sand culture instead of being transferred to distilled water. Each group was divided into inoculated and control plants. Inoculations were made at the same time using the same inoculum. Both permeability and respiratory change determinations were made at 12-hour intervals after inoculation. Permeability measurements were made as described before. For respiration measurements, representative plants were removed from the sand, and the respiratory rate of 500 mg root samples was determined. Total nitrogen of the root samples was determined at the end of the experiments and the oxygen uptake per mg N per hour was calculated. No change in the respiratory rate was obtained up to the time the permeability change first became pronounced (Fig. 7B). From 12-24 hours later a decrease in the respiratory rate of roots of inoculated plants was obtained. The oxygen uptake continued to decrease steadily thereafter and was about 50% of that of the control at the first day of wilt. Thus, no increase in respiration was detected in these experiments.

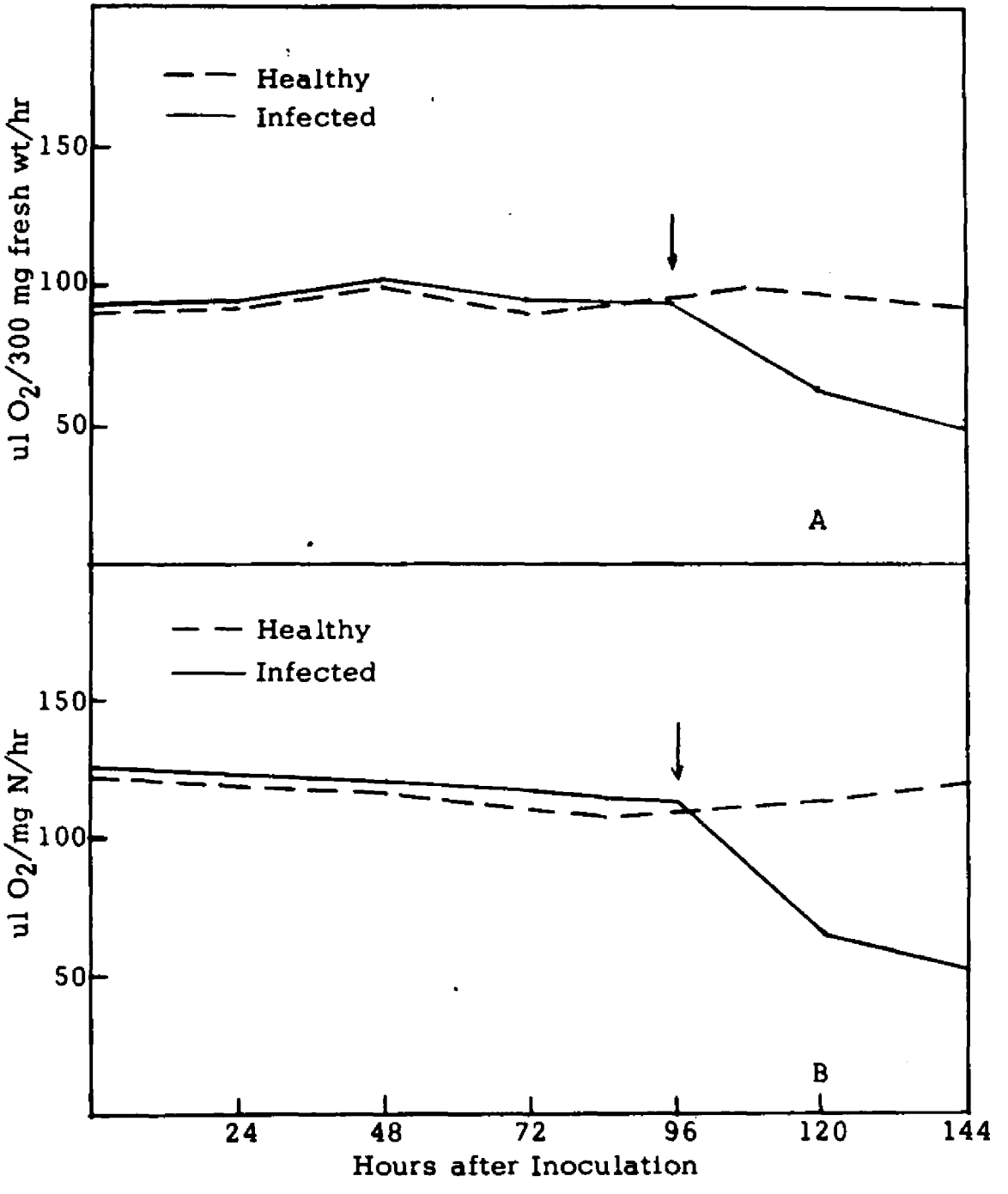

Figure 7. Changes in the respiratory rates of roots of TEV-inoculated and control Tabasco pepper plants during the 144 hours after inoculation. The arrow refers to the time the initial change in permeability was detected. A. Roots were suspended in distilled water before respiratory determination. B. Roots were harvested from plants grown in sand culture.

The effect of KCl on respiration of TEV-inoculated Tabasco pepper plants was also studied. Roots suspended in distilled water, and in KCl solutions of 10^{-2}, 10^{-3}, and 10^{-4}M were used for respiratory rate measurements. Roots suspended in distilled water were used for parallel determinations of permeability change. Permeability measurements were made at 24-hour intervals. At the time permeability change first became pronounced, root samples were collected and the oxygen uptake per 500 mg fresh wt per hour was measured.

As shown in Table 3 an increase in the respiratory rates of roots of inoculated plants in 10^{-2}M KCl was obtained. The increase in the respiratory rate was more pronounced 12 hours after the initial change in permeability. Roots suspended in distilled water showed a decrease in the respiratory rate which was apparent 48 hours after the permeability changes first became pronounced. In the case of 10^{-3}M KCl the data from healthy and inoculated plants overlapped, and the averages were very close. The 10^{-4} KCl treatment gave results comparable to the water treatment. It was noted that roots from healthy plants suspended in KCl solutions had lower respiratory rates than those suspended in water.

The changes in the respiratory rates of roots of healthy and TEV-inoculated California Wonder pepper plants were determined. No changes in the respiratory rates were obtained during 144 hours after inoculation as shown in Figure 8.

Table 3. The effect of 3 concentrations of KCl on the respiratory rates of roots of healthy and TEV-inoculated Tabasco pepper plants.

Exp.	Treatment	ul O_2/500 mg fresh wt/hour[a]			
		12 hours[b]		48 hours[b]	
		Healthy	Inoculated	Healthy	Inoculated
1	H_2O	200	180	197	126
	KCl (10^{-2}M)	164	230	145	163
	KCl (10^{-3}M)	200	215	157	148
	KCl (10^{-4}M)	203	198	150	110
2	H_2O	230	220	250	187
	KCl (10^{-2}M)	138	170	130	140

[a]Duplicate or triplicate samples were used for each treatment.

[b]Experiments were made 12 and 48 hours after the initial change in permeability.

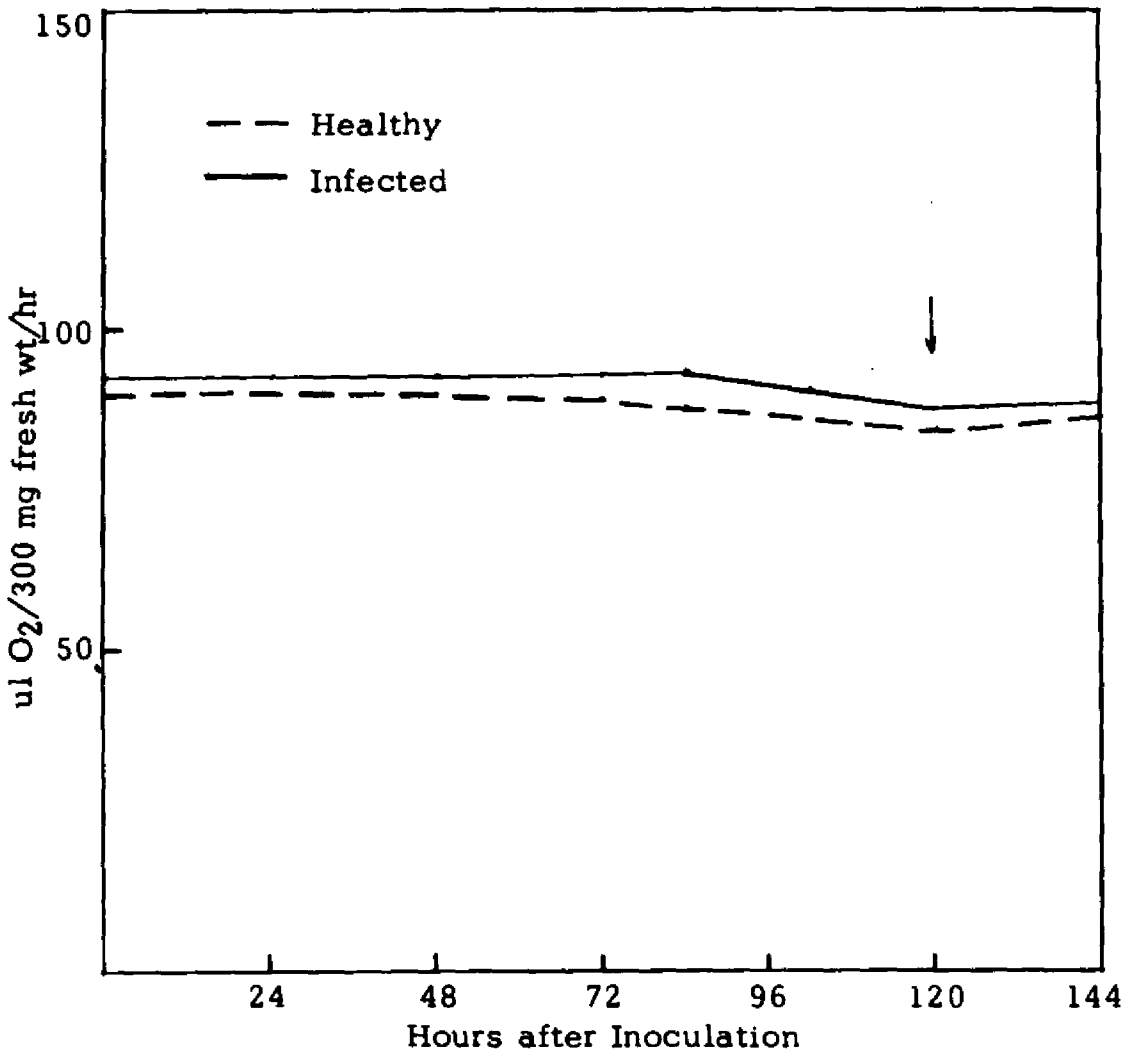

Figure 8. Changes in the respiratory rates of roots of healthy and TEV-infected California Wonder pepper plants during the 144 hours after inoculation. The arrow refers to the time of symptom appearance on young leaves.

Changes in the respiratory rates of healthy and TEV systemically infected Tabasco pepper leaves also were studied. Results obtained from these experiments showed that a 40% increase in oxygen uptake of systemically infected leaves was detected at the time mottling of the young leaves occurred (Fig. 9). This higher rate of respiration was maintained thereafter. The respiratory rates of healthy and systemically infected leaves of California Wonder and Cayenne pepper plants were determined at the time vein clearing and mottling symptoms of the young leaves developed. Tabasco pepper plants were included in these experiments for comparison. An increase in the respiratory rates of systemically infected leaves was found to be the case with all 3 varieties (Table 4). No change in the respiratory rates of the leaves could be detected before symptom appearance in any of the 3 varieties.

Effect of TEV infection on ascorbic acid content

Ascorbic acid content of roots of TEV-infected and healthy Tabasco pepper plants was determined at the first day of wilt. As shown in Table 5, a sharp decrease in ascorbic acid content was obtained with infected roots. Ascorbic acid content in systemically infected leaves, which were collected from the same plants used for root analysis, did not differ from that in corresponding leaves from healthy plants (Table 5).

The effect of TEV infection on ascorbic acid content in roots of California Wonder and Cayenne pepper varieties was studied as a

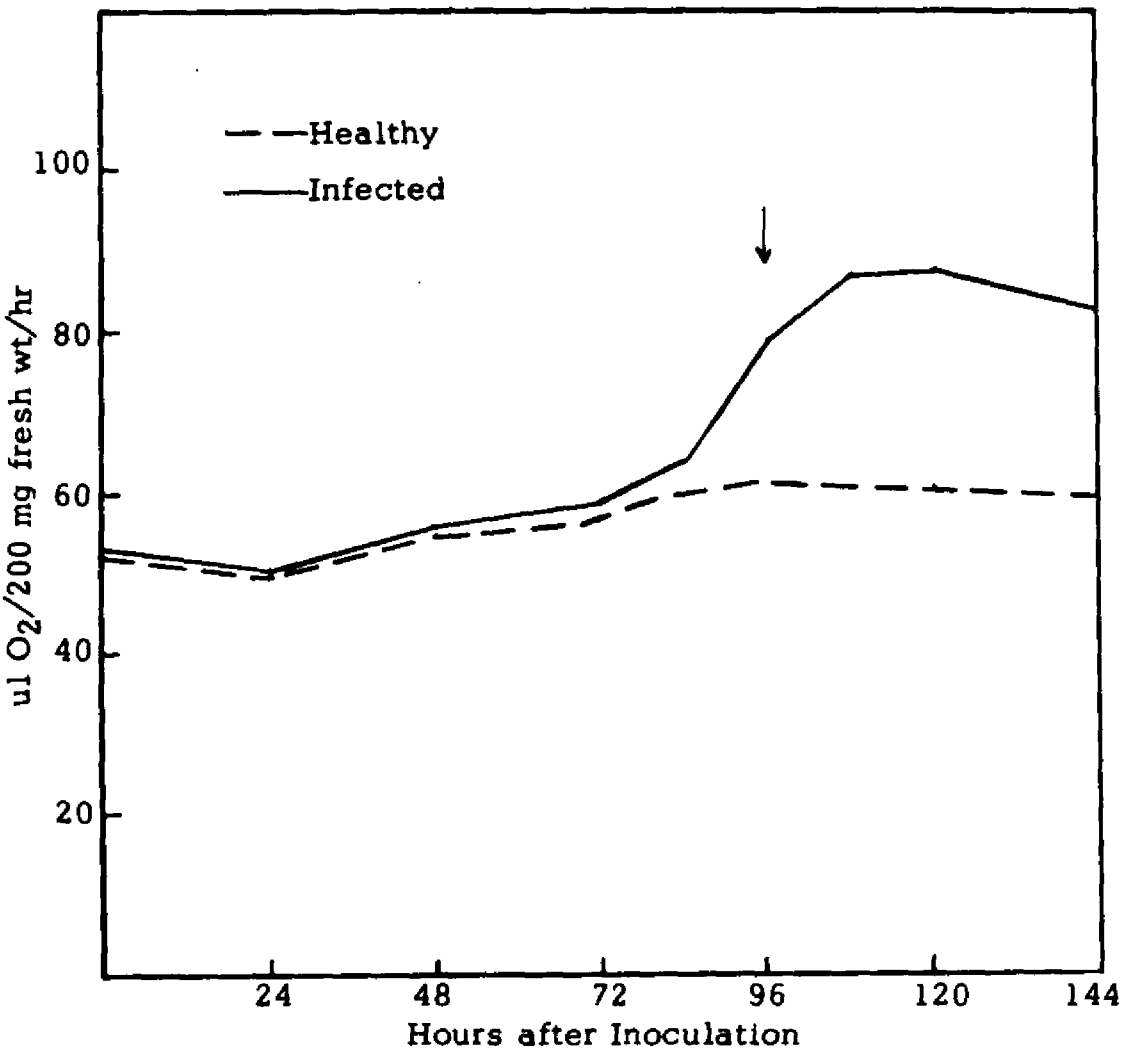

Figure 9. Changes in the respiratory rates of healthy and systemically infected leaves of Tabasco pepper plants during the 144 hours after inoculation. The arrow refers to the time of symptom appearance on young leaves.

Table 4. Respiratory rates of healthy and TEV systemically infected leaves of 3 pepper varieties.[a]

Variety	ul O_2/hr /200 mg fresh wt		ul O_2/hr /mg dry wt	
	Healthy	Infected	Healthy	Infected
Cayenne[b]	86	116		
California Wonder[b]	75	103		
Tabasco[c]	81	106	2.1	2.7

[a]Each figure represents an average of 3 replications. Experiments were done twice.

[b]Determinations were made at the time vein clearing and mottling symptoms of the young leaves were apparent.

[c]Leaves were drooping.

Table 5. Ascorbic acid content of roots of healthy and TEV-infected plants of 3 pepper varieties.[a]

Variety	Ascorbic acid[b] ug/g fresh wt Healthy	Infected
Cayenne (roots)	58	56
California Wonder (roots)	53	57
Tabasco (roots)	70 (54)[c]	34 (24)[c]
Tabasco (leaves)	980	1000

[a]Each figure represents the average of 10 determinations.

[b]Determinations were made with the 2,4-dinitrophenyl hydrazine reagent.

[c]Determinations were made with the indicator 2:6-dichlorophenol-indophenol.

further control. Root samples were harvested 8 days after inoculation, at which time vein clearing and mottling symptoms were apparent on the young leaves. Results obtained from these experiments (Table 5) indicated no difference in the level of ascorbic acid between healthy and infected plants of either variety.

These experiments were repeated twice with the same results.

Effect of TEV infection on polyphenol content

Examination of the chromatograms prepared from root extracts of TEV-infected and healthy Tabasco pepper plants 6 and 9 days after inoculation, showed chlorogenic acid to be the most prominent spot on the sheets. The intensity of the blue fluorescent spot produced by chlorogenic acid in ultraviolet light was greater with root extracts from infected plants than with the corresponding extracts from healthy plants. A 75% increase in chlorogenic acid content was detected in root extracts of TEV-infected plants at the first day of wilt (Table 6). Still higher values for chlorogenic acid could be detected 3 days after wilt had appeared (Table 6). A comparable increase in the total orthodihydric phenols was obtained with root extracts from infected plants (Table 6).

As a second control, chlorogenic acid and total orthodihydric phenols were determined in the roots of TEV-infected and healthy Cayenne pepper plants. As shown in Table 6, no such increase in the level of polyphenols was detected in the roots of infected plants.

Table 6. Polyphenol content of roots of healthy and TEV-infected plants of 2 pepper varieties.[a]

	Date of assay after inoculation	Chlorogenic acid[d] mg/g fresh wt		Orthodihydric phenols[e] mg/g fresh wt	
		Healthy	Diseased	Healthy	Diseased
Tabasco	6[b]	.290	.508	.330	.550
	9	.290	.660	.320	.830
Cayenne	8[c]	.250	.275	.300	.340

[a]Each figure represents the average of 5 determinations.

[b]Plants were starting to wilt.

[c]Symptoms of vein clearing and mottling were apparent on young leaves.

[d]Determined by paper chromatographic separation followed by ultraviolet spectrophotometric assay.

[e]Determined by the Arnow reagent in chlorogenic acid equivalents.

Changes in the activity of some soluble oxidases

The activity of polyphenol oxidase was determined manometrically using chlorogenic acid as a substrate. No endogenous oxygen uptake was observed in any of the tests. The highest rate of enzymatic activity was obtained in all cases in the first 10 minutes after tipping chlorogenic acid into the flasks, after which the rate of the reaction leveled off (Fig. 10). Comparisons of the enzymatic activity of the different root extracts therefore, were made on the basis of oxygen consumption in the first 10 minutes after tipping. As shown in Table 7, no differences in the activity of polyphenol oxidase were observed between root extracts of healthy and TEV-infected plants of any of the 3 pepper varieties.

The enzymatic oxidation of ascorbic acid was also studied manometrically. An increase in the enzymatic oxidation of ascorbic acid was obtained with root extracts of TEV-infected Tabasco pepper (Table 8). No differences were obtained with the other pepper varieties.

Peroxidase activity was found to be greatly enhanced in root extracts of TEV-infected Tabasco pepper plants at the first day of wilt. No such increase in peroxidase activity was observed with other pepper varieties, at the time the young leaves showed vein clearing and mottling symptoms (Fig. 11).

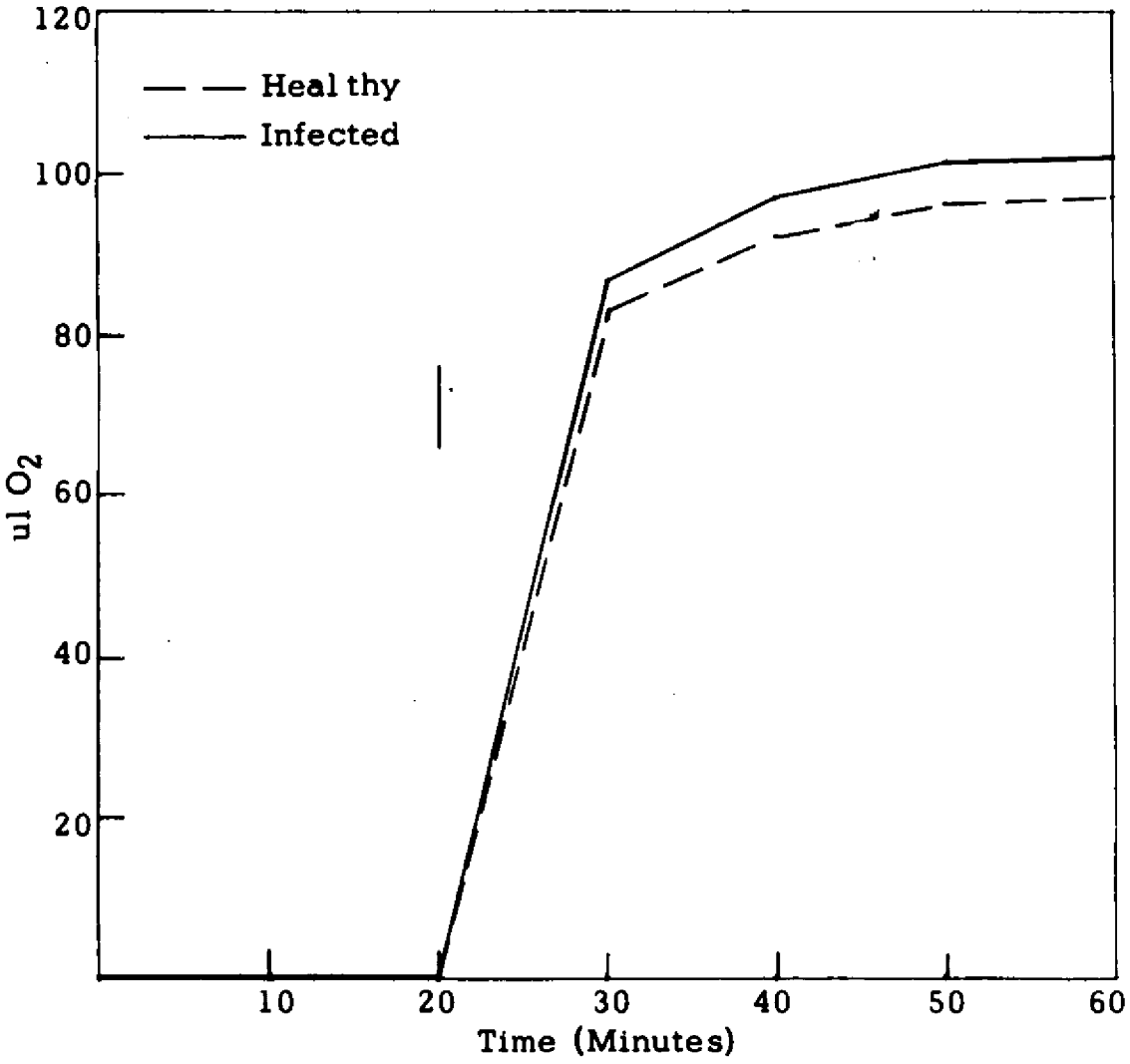

Figure 10. Polyphenol oxidase activity of root extracts of healthy and TEV-infected Tabasco pepper plants. Determination was made at the first day of wilt.

Table 7. Polyphenol oxidase activity of root extracts of healthy and TEV-infected plants of 3 pepper varieties.[a]

Variety	ul O_2/10 min[b]	
	Healthy	Infected
Tabasco[c]	83	86
Cayenne[d]	56	53
California Wonder[d]	58	61

[a]Enzyme activity of an extract corresponding to 600 mg fresh wt root tissue.

[b]Each figure is an average of 3 samples. Duplicate flasks were used for each sample.

[c]Determinations were made at the first day of wilt.

[d]Determinations were made at the time vein clearning and mottling symptoms of the young leaves became apparent.

Table 8. Enzymatic oxidation of ascorbic acid by root extracts of healthy and TEV-inoculated plants of 3 pepper varieties.[a]

	ul O_2/40 min[b]			
	Healthy		Infected	
Variety	Nontreated	Boiled Extract[c]	Nontreated	Boiled Extract[c]
Tabasco[d]	117	41	164	46
Cayenne[e]	61	40	55	30
California Wonder[e]	91	39	88	35

[a]Enzyme activity of an extract corresponding to 600 mg fresh wt root tissue.

[b]Each figure is an average of 3 samples. Duplicate flasks were used for each sample.

[c]Extract was boiled for 2 minutes.

[d]Determinations were made at the first day of wilt.

[e]Determinations were made at the time vein clearing and mottling symptoms of the young leaves became apparent.

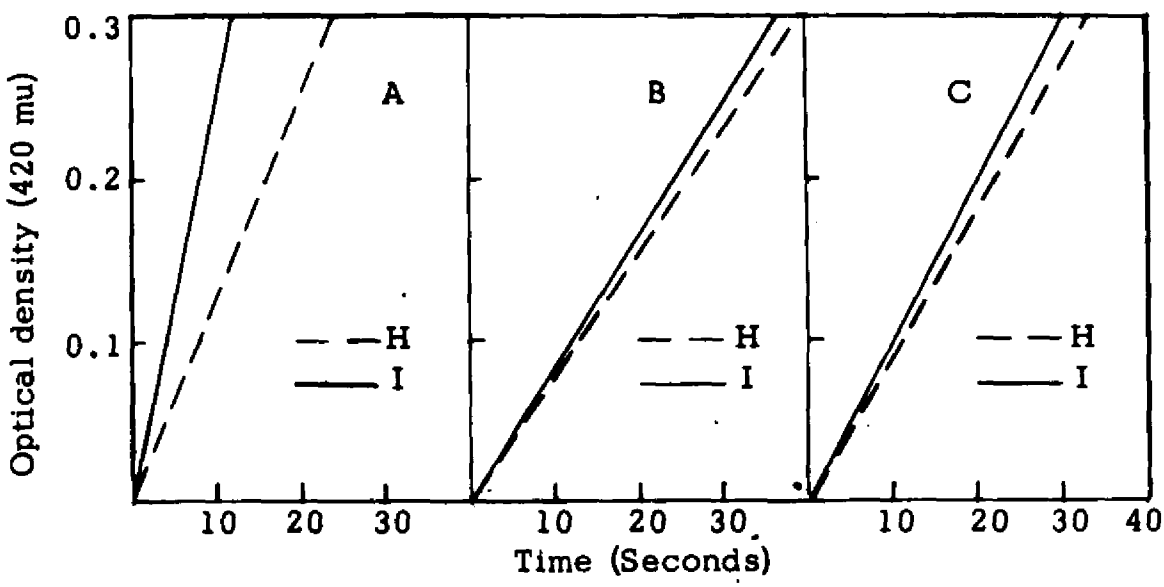

Figure 11. Peroxidase activity of root extracts of healthy and TEV-infected plants of the 3 pepper varieties. A. Tabasco pepper; B. Cayenne; C. California Wonder. (H = healthy; I = infected)

DISCUSSION

Attempts to remove the inhibitor of TEV infection of Chenopodium sp. either failed or resulted in large losses of virus.

Since the local-lesion technique was not applicable, estimation of virus concentration in the root extracts of the 3 pepper varieties was based on systemic infection. The infectivity of these root extracts was found to be quite similar (Table 2) and these results suggest that the rate of virus multiplication in the roots of the 3 pepper varieties does not differ significantly. Since it is unlikely that small differences in virus titer could account for the unique behavior of Tabasco pepper, the possibility that this unique response is due to differences in virus concentration is ruled out.

Virus diseases, in general, would seem to be good systems for studies on the effect of infection upon host metabolism since no metabolic activity has been associated with isolated viruses. This offers an advantage over fungal and bacterial diseases in which 2 metabolic systems, those of the host and the pathogen, are involved. Since Tabasco pepper is the only variety among the TEV-susceptible pepper varieties which develops wilt as a result of TEV infection and TEV is the only virus which causes such symptoms on Tabasco pepper this disease provides an excellent system for studies of the physiology and biochemistry of wilt diseases.

A considerable loss of electrolytes, indicative of permeability change, was the earliest response to TEV infection detected in the roots of Tabasco pepper plants. Permeability changes were found to precede the respiratory changes as well as histological and wilt symptoms. Since permeability alteration was not observed in roots of noninoculated control plants or in roots of TEV-susceptible pepper varieties which do not wilt as a result of infection, it is tempting to suggest a causal relationship between the increased permeability of the roots and the wilting which follows later on.

Alteration of the permeability of root cells was listed by Subermanian and Saraswathi-Devi (51) as a possible mechanism of pathological wilt. The altered permeability of the membranes would bring about changes in the osmotic gradient, thus influencing the osmotic movement of water (11). In view of the current knowledge of the mechanisms of water absorption, the osmotic water movement is strongly emphasized (28). Of special interest in this respect is Gäumann's (12) work on fusaric acid, a toxic product of the wilt fusaria. Gäumann (12) emphasized the role of permeability changes in relation to the injurious effects of fusaric acid. Sadasivan (47) suggested a mechanism of wilt based on the impairment of the permeability of root cells by fusaric acid. He surmized that the first damage to tissue is impairment of permeability followed by loss in conductivity of sap and consequently a loss of turgor and imbalance

of the ionic status of the effected cells. The other *in vivo* physiological and biochemical changes associated with wilted plants appear to be the sequelae of the earlier damage. The role of fusaric acid in fusarial wilts, however, has been questioned since fusaric acid does not produce all symptoms of the disease and shows no host specificity (58).

The recovery of wilted Tabasco pepper plants when placed in water after the excision of their root tips could be explained as follows: In the intact wilted plant, the root cells which have lost their turgor as a result of the altered permeability of the membranes, as well as the necrotic phloem and cambium cells, would probably represent a barrier to water transport from the root surface to the xylem. Upon excision of the root tips, water becomes available to the xylem vessels and can again be taken up by the plant. This explanation is consistent with Philip's (44) statement that the zone between the root surface and the xylem is one of 3 significant segments in the water transport pathway where resistance to flow is likely to be important.

The data on qualitative and quantitative determinations of the substances that leach out from the roots of inoculated Tabasco pepper plants indicated that K was the major ion that was released as a result of altered permeability of the membranes. This finding leads to the question of whether the loss of K is related to phloem necrosis and wilt development since K loss precedes the other two phenomena. Hartt's (17) observation that K deficiency caused phloem necrosis in

sieve tubes and companion cells of sugarcane might be of interest in this respect. A marked release of K ions from cut shoots of tomatoes was observed by Linskens (32) within a few hours after treatment with fusaric acid. Furthermore, Sadasivan and Kalyanasundaram (48) have studied changes in levels of various inorganic ions in cotton plants naturally infected with Fusarium vasinfectum. The outstanding change found was a large decrease in the K content of diseased plants. These results would seem comparable with the finding that K was the predominant ion that was leached out of the roots of TEV-infected Tabasco pepper plants.

The decrease in the respiratory rates that follows permeability changes in the roots of TEV-infected Tabasco pepper plants suspended in water might be explained on the basis that the altered permeability of the membranes allows substrates and cation activators required for the activity of the respiratory enzymes to diffuse into the surrounding solutions. Paquin and Waygood (39) explained the inhibition of the respiratory enzymes of tomato hypocotyls by fusaric acid as resulting from an impairment of the semipermeability of the mitochondrial membrane so that cytochrome-c escaped and was thus removed from the site of action.

Experiments in which roots were suspended in KCl concentrations of 10^{-3} and 10^{-4}M gave variable results. However, a consistent increase in respiration was obtained with roots of infected Tabasco pepper suspended in 10^{-2}M KCl over the control plants in the same

concentration of KCl. A clear-cut conclusion cannot be drawn from these results since various possibilities have to be considered. The increase in the respiratory rates of infected roots suspended in 10^{-2}M KCl may be explained on the basis that KCl in the outer solutions alleviates the loss of K which occurs in comparable roots suspended in water. However, other possible effects of the KCl must be considered in view of the observed inhibition of respiratory rates of healthy roots in KCl. Amador and Wheeler's (3) work on the effect of leaching on the respiration of victorin-treated oat tissues is of importance in this respect, since victorin is known to induce permeability changes. The duration of elevated respiration induced by victorin was markedly reduced by leaching in distilled water. Amador (2), in further studies, found that the leaching effect could be partially nullified when victorin-treated tissues were leached in 10^{-1}M KCl solutions.

The increase in the respiratory rates of systemically infected leaves of Tabasco pepper is apparently a typical response to TEV infection since such an increase was also observed with the other pepper varieties (Table 4). The fact that respiratory rates of roots of California Wonder plants did not change as a result of infection, whereas an increase in the respiration of systemically infected leaves was obtained, points out that different tissues differ in their response to virus infection.

A decrease in ascorbic acid content was detected in the roots of TEV-infected Tabasco pepper plants at the first day of wilt.

Ascorbic acid is normally maintained in a reduced state in the plant cells and as a result, the concentration of its oxidation product, dehydroascorbic acid (DHA), is usually very small (35). An upset of this balance would cause a rapid conversion of ascorbic acid to DHA. Mapson (35) has listed a) mechanical damage, b) the action of substances causing cellular disorganization, and c) the action of specific enzymatic poisons, as possible disturbing factors. Since TEV infection of Tabasco pepper altered the permeability of the root cells thus disturbing cellular organization, it is possible on this basis to explain the decrease in ascorbic acid content. The increased enzymatic oxidation of ascorbic acid which was detected in root extracts of infected plants at the first day of wilt is in agreement with the finding that ascorbic acid content had decreased (Tables 5, 8). The finding that ascorbic acid content and its enzymatic oxidation were not changed in roots of the other pepper varieties (Table 5) offers further evidence that permeability changes may cause changes in ascorbic acid content. In addition, permeability changes were not detected in systemically infected Tabasco leaves and ascorbic acid content did not change.

Krupka's (29) work on the effect of victorin on ascorbic acid content and the enzymatic oxidation of ascorbic acid in oat tissue is of extreme interest since victorin is known to induce permeability changes (56). Krupka (29) found a decrease in ascorbic acid content and 3-fold increase in the oxidation of ascorbic acid.

A rapid decrease in ascorbic acid was also reported by Johnson and Schaal (25) in potato tubers mechanically damaged or those showing pronounced net necrosis caused by aster yellows virus infection.

Reid (45) suggested that ascorbic acid would seem to function at the surface of the root cells as a regulator of water intake and its retention. It would be interesting if the decrease in ascorbic acid is related to wilting on the basis of this suggestion.

The accumulation of polyphenols in the roots of TEV-infected Tabasco pepper plants is not surprising since an increased aromatic biosynthesis is characteristic of all injured or infected tissues. The browning of the root tissues associated with the wilted plants indicates a type of hypersensitive response of root tissues to virus infection. The browning of such tissues has always been attributed to oxidation products of polyphenols (9). The evidence that these phenols undergo oxidation in Tabasco roots is indirect and is based on the decrease in ascorbic acid content and enhanced peroxidatic activity. Ascorbic acid was probably used up in maintaining the phenol in the reduced state.

It might seem surprising that in spite of the increased polyphenol synthesis, the <u>in vitro</u> activity of polyphenol oxidase in root extracts of infected plants was not different from that of healthy plants. This could be misleading since phenol oxidases are generally inactive in intact tissues and the amount of activity in extracts is, therefore, not an indication of the activity in the tissue (1). Damage to the cell in

infected tissues, as a result of impairment of permeability, would allow the oxidases to come into contact with their substrate, thus resulting in a greatly increased rate of oxidation. This would not be the situation in intact healthy tissue. If we assume that polyphenol oxidation products are responsible for the browning of tissue, it might also seem contradictory that the polyphenol content increased steadily rather than decreased. Farkas and Kiraly (9) emphasized that a decrease in polyphenol is not a general phenomenon characteristic of all hypersensitively reacting tissues. For example, in wheat leaves reacting hypersensitively to rust infection, the phenol content increases steadily (26). It is of interest in this respect to refer to Johnson and Schaal's (23, 25) finding that potato tubers showing pronounced net necrosis caused by aster yellows virus infection accumulate orthodihydric phenols in the necrotic areas. Hirai (18) has also reported increased polyphenol synthesis in and around the necrotic cells of potato leaves infected by potato virus X or Y.

The peroxidatic activity of root extracts of infected plants was greatly enhanced. Since peroxidases may act on essentially the same substrate as polyphenol oxidases (8), it is very tempting to attribute to them a similar role in host-pathogen relationships. Demonstration of the presence of H_2O_2, the substrate for peroxidase, in the Tabasco pepper roots is essential before peroxidase is assigned a role in polyphenol oxidation.

A number of workers have reported enhanced peroxidase activity in virus-infected tissues (33, 53, 63). Mace (34) showed that peroxidase from Fusarium-infected banana roots is capable of catalyzing the oxidation of dopamine, thus indicating that peroxidase may participate in the in vivo oxidation of dopamine to produce vascular browning. In histochemical tests of banana roots, Mace (34) demonstrated that peroxidase occurred as cytoplasmic particles in parenchyma cells of phloem bundles. Furthermore, Van Fleet (54, 55) reported the presence of peroxidase in all kinds of phloem cells: sieve elements, companion cells, and surrounding parenchyma cells.

The localization of peroxidase in the phloem is interesting in view of the enhanced peroxidase activity detected in infected Tabasco roots at the first day of wilt and the phloem necrosis prominent at this time.

SUMMARY

1. Attempts made to eliminate the virus inhibitor present in pepper extracts either failed or resulted in large losses of virus.
2. The virus titer, based on systemic infection, of root extracts of the 3 pepper varieties was found to be quite similar.
3. A marked release of electrolytes, indicative of permeability change, was the first response that could be detected in the roots of TEV-infected Tabasco pepper plants. This occurred 24-48 hours before wilt symptoms. No such loss of electrolytes was obtained with roots of noninoculated control plants or with roots of other TEV-susceptible pepper varieties which do not wilt as a result of infection.
4. Potassium and sodium ions were the major electrolytes that were released into the surrounding solutions as a result of the altered permeability of the root cells.
5. No histological changes were observed at the time the initial change in permeability was detected. At the first day of wilt, however, cambium and phloem necrosis was very characteristic in root sections of wilted plants.
6. No changes in the respiratory rates of the roots were detected up to the time the permeability changes first became pronounced. However, 12-24 hours later, a decrease in the oxygen uptake by roots of infected Tabasco pepper plants was observed. The

respiratory rate continued to decrease steadily thereafter, and was about 50% of that of the control at the time of incipient wilt. No significant changes in the respiratory rates of roots of TEV-infected California Wonder plants were observed during 144 hours after inoculation.

7. TEV-infected Tabasco pepper plants whose roots were suspended in 10^{-2}M KCl solutions showed a higher respiratory rate than roots of corresponding healthy plants in the same solutions.

8. Systemically infected leaves of the 3 pepper varieties showed a higher respiratory rate than corresponding leaves of healthy plants at the time the external symptoms were apparent.

9. A decrease in ascorbic acid content was detected in the roots of TEV-infected Tabasco pepper plants at the first day of wilt. No such decrease was obtained with systemically infected leaves or with roots of TEV-infected California Wonder and Cayenne pepper plants.

10. An increased enzymatic oxidation of ascorbic acid was detected in root extracts of TEV-infected Tabasco pepper plants at the first day of wilt. No differences between root extracts of healthy and infected plants were obtained with the other pepper varieties.

11. A pronounced accumulation of chlorogenic acid and orthodihydric phenols was detected in the roots of TEV-infected Tabasco pepper plants. No such increase in polyphenols was detected in roots of diseased Cayenne pepper plants.

12. No significant changes in the activity of polyphenol oxidase were obtained with root extracts of any of the 3 pepper varieties.

13. A greatly enhanced peroxidase activity was found in TEV-infected Tabasco pepper root extracts at the first day of wilt. No such increase in the peroxidatic activity was observed with other pepper varieties.

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VITA

Said Amin Ghabrial was born on October 1, 1939 in Cairo, Egypt. He received his elementary and secondary school education in Cairo. In 1955 he enrolled in Cairo University, College of Agriculture, where he received his B.Sc. in June 1959. He worked as an Agricultural Engineer for the United Arab Republic Government, Ministry of Land-Reform from November 1959 until he was inducted in the Army of the United Arab Republic in March 1961. He was discharged from the army when he received a scholarship from the United Arab Republic Government in September 1961 to study plant pathology in the United States at Louisiana State University. He received the Master of Science degree in Plant Pathology in June 1963. He is now a candidate for the degree of Doctor of Philosophy in Plant Pathology in June 1965.

EXAMINATION AND THESIS REPORT

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Major Field: Plant Pathology

Title of Thesis: Physiological and Biochemical Changes Associated with Virus-Induced Wilt of Tabasco Pepper

Approved:

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Date of Examination:

April 27, 1965